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Sodium-chloride-induced protection in nephrotoxic acute renal failure: Independence from renin

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Sodium-chloride-induced protection in nephrotoxic acute renal failure: Independence from renin. It has been shown that the severity of experimentally induced acute renal failure (ARF) is inversely related to dietary sodium chloride intake, and the effects have been attributed to the concurrent changes in renal renin. In the current study, renal renin of rats was increased by chronic sodium deprivation and decreased by chronic sodium loading and DOCA administration. In two nephrotoxic models (mercuric chloride, uranyl nitrate), giving previously sodium-deprived rats 1% sodium chloride to drink for 48 hours prior to ARF induction greatly attenuated the severity without any reduction in their high renal renin. Conversely, giving previously sodium-loaded rats tap water to drink for 4 to 5 days prior to AFR induction greatly enhanced the severity without any increase in their subnormal renal renin. Therefore, the changes in severity of ARF resulting from changes in dietary sodium are not mediated by changes in renal renin. Significant inverse correlations were found between mean peak BUN values during the follow-up period (5 to 7 days) and the 24-hour urinary sodium excretions prior to ARF induction in both models, suggesting that sodium intake and/or excretion at the time of induction is a good predictor of the severity. The effects of sodium chloride in both models were predominantly expressed during the maintenance phase, and consisted of attenuation of the severity (both models) and hastening of the recovery (mercuric chloride model). Possible mechanisms by which dietary sodium produced its effects, independently of its effects on the renin-angiotensin system, are discussed.

Effet protecteur de chlorure de sodium vis-à-vis de l'insuffisance rénale aiguë néphrotoxique: Indépendance de la rénine. Il a été montré que la sévérité de l'insuffisance rénale aiguë expérimentale (ARF) est en proportion inverse de la charge alimentaire en le chlorure de sodium, et cet effet a été attribué aux modifications de la rénine rénale. Dans ce travail la rénine rénale a été, chez des rats, augmentée par une déplétion chronique en sodium et diminuée par une charge chronique en chlorure de sodium et de la DOCA. Dans deux modèles de néphrotoxicité (chlorure mercurique et nitrate d'uranyle) le fait de donner aux animaux préalablement déplétés en sodium du chlorure de sodium 1% comme boisson pendant 48 heures avant l'induction de l'ARF a atténué la sévérité de celle-ci sans modifier la concentration élevée de rénine rénale. A l'inverse, le fait de donner à des rats préalablement chargés en sel de l'eau du robinet comme boisson pendant 4 ou 5 jours avant l'induction de l'ARF augmente considérablement la sévérité de celle-ci sans élever la concentration, inférieure à la normale, de rénine rénale. Ainsi les modifications de la sévérité de l'ARF consécutives aux modifications de l'apport alimentaire de sodium n'ont pas la rénine rénale comme médiateur. Des relations inverses significatives ont été observées entre l'azote sanguin non protéique pendant la période d'observation (5 à 7 jours) et la natriurèse de 24 heures avant l'induction de l'ARF dans les deux modèles, ce qui suggère que l'apport alimentaire et/ou l'excrétion de sodium au moment de l'induction d l'ARF sont un bon élément de prévision de la sévérité. Les effets de chlorure de sodium dans ces modèles sont surtout exprimés au cours de l'évolution et consistent en une diminution de la sévérité, dans les deux modèles, et une récupération plus précoce, dans le modèle où le chlorure mercurique est utilisé. Les mécanismes par lesquels le sodium alimentaire produit ces effets, indépendamment des effets sur le système rénine-angiotensine, sont discutés.

It has been shown that if rats are given 1% sodium chloride or 2% potassium chloride to drink for several weeks, rather than tap water, they are protected against acute renal failure (ARF) induced by the administration of glycerol, mercuric chloride, dichromate, or uranyl nitrate [1–6]. This protective effect of chronic salt loading has been attributed to depletion of renal cortical renin content (RCRC) [1, 4–6]. Moreover, chronic sodium restriction, which increases RCRC [7], enhances the severity of glycerol-induced ARF in rats [8]. Although no cause-and-effect relationship between RCRC and severity of ARF has been established, these observations are consistent with the hypothesis that RCRC is directly related, in a nearly proportional manner, to the severity of experimentally-induced ARF [4, 6].

Thiel et al [9] have shown that protection in the mercuric chloride model can be achieved without suppression of renal renin. We have shown that protection in the glycerol model antedates any detectable suppression of renal renin during the course of salt loading [10]. Although RCRC was not

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detectably suppressed in either of these studies, it is conceivable that renin activity at a critical intrarenal site was decreased, mediating the protective effects. Thus, a clear dissociation of the effects of salt loading on the renin-angiotensin system and on the severity of ARF has not been demonstrated.

The present study was designed to examine the possible independent effects of changes in sodium chloride intake on RCRC and on the severity of mercuric-chloride- and uranyl-nitrate-induced ARF in rats.

Methods

Female Sprague-Dawley rats weighing between 175 and 300 g were used in this study. The experimental protocol is outlined in Fig. 1. Group-A rats (normal RCRC) were given tap water and Purina Lab Chow. In groups B and C (low RCRC), 1% sodium chloride was substituted for tap water, and each rat received 10 mg of DOCA/kg body wt, s.c., four to five times per week for 5 to 6 weeks. Four to five days before the experiments, the DOCA injections were stopped, and tap water was substituted for sodium chloride in group C. In groups D, E, and F (high RCRC), a low-sodium diet ("Rat Modified," Nutritional Biochemicals, Cleveland, Ohio) was given for 5 to 6 weeks. Four to five days before the experiments, group E was switched to the normal chow. It was expected that groups C and E would be similar with respect to salt balance, but different with respect to RCRC, at the time of induction of ARF. To determine if protection could be achieved despite high RCRC, we gave group F 1% sodium chloride to drink for 48 hours before ARF induction. The numbers of animals in each group and subgroup can be found in Tables 1 and 2.

All animals were kept in metabolic cages, and fluid intake and urine output were monitored for the 24 hours before the experiments. Urine was analyzed for sodium, potassium, and chloride as described below. Then, random animals from groups A to F were sacrificed for determination of RCRC and plasma renin concentration (PRC). Rats were decapitated, and blood was collected within 10 to 15 seconds in chilled, heparinized tubes. These were centrifuged at 4° C, and the plasma was frozen until analysis. Kidneys were removed, put in iced 150 mM sodium chloride, and stored frozen until analysis.

Tail-vein blood samples were obtained from the rest of the animals in groups A to F for baseline BUN determinations. Then ARF was induced by subcutaneous injection of either mercuric chloride (4.7 mg/kg body wt) or uranyl nitrate (5 mg/kg body wt). To follow the course of ARF, we determined BUN concentrations (tail-vein blood sample) on days 1, 2, 3, 5, and 7 after the injections. Dietary regimens during this time were the same as those in force at the time of ARF induction. At the end of the follow-up period, enough blood was collected from some of the sacrificed rats for simultaneous determinations of BUN and serum creatinine concentrations.

To determine the PRC response to mercuric chloride, we sacrificed additional animals of groups A to F by decapitation 6 hours after injection. In these

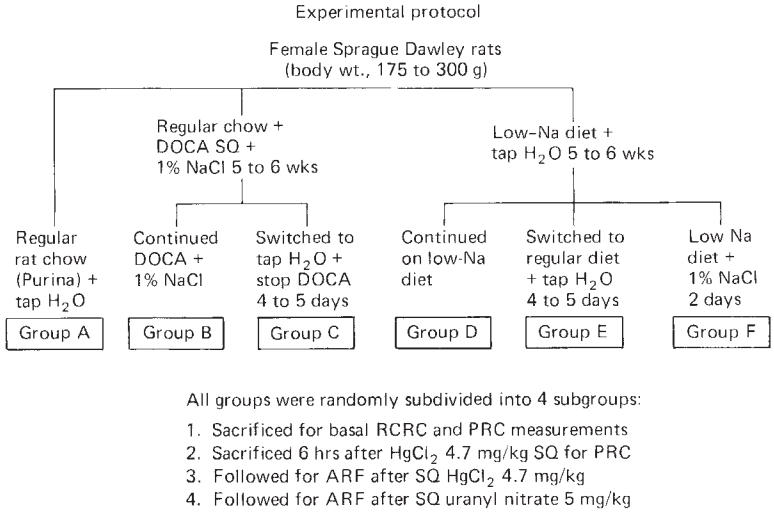

Fig. 1. *Experimental protocol.* Renal cortical renin content (RCRC) of female Sprague Dawley rats varied between low (groups B and C) and high (groups D to F). Short-term changes in sodium intake were imposed as indicated. PRC is plasma renin concentration; ARF is acute renal failure.

Table 1. Baseline BUN and 24-hour excretion data, groups A to C[a]

	BUN *mg/100 ml*	V *ml/24 hr/100 g body wt*	$U_{Na}V$	$U_{Cl}V$	U_KV
			μEq/24 hr/100 g body wt		
Group A					
RCRC (9)	15 ± 1	7 ± 1	274 ± 59	270 ± 60	532 ± 119
PRC (8)	—	4 ± 1	227 ± 41	251 ± 50	432 ± 113
$HgCl_2$ (13)	14 ± 1	6 ± 1	334 ± 41	433 ± 69	721 ± 82
UN (11)	15 ± 1	4.0 ± 0.4	233 ± 40	281 ± 46	474 ± 71
Group B					
RCRC (11)	13 ± 1	26 ± 3	5191 ± 562	5121 ± 562	825 ± 95
PRC (9)		28 ± 2	5593 ± 422	5469 ± 440	455 ± 104
$HgCl_2$ (9)	19 ± 3	28 ± 3	5815 ± 460	5973 ± 499	899 ± 196
UN (12)	18 ± 1	27 ± 2	5903 ± 380	5228 ± 382	393 ± 57
Group C					
RCRC (11)	14 ± 2	8 ± 1	466 ± 74	520 ± 90	842 ± 140
PRC (8)	—	6 ± 1	494 ± 57	555 ± 75	950 ± 118
$HgCl_2$ (10)	14 ± 1	7 ± 1	496 ± 70	565 ± 87	828 ± 105
UN (10)	15 ± 2	7 ± 1	539 ± 80	574 ± 112	884 ± 153

[a] Values are means ± SEM. Number of animals per subgroup is in parentheses. Refer to text or Fig. 1 for treatment of groups A, B, and C. Subgroups are: RCRC, rats sacrificed for determination of renal cortical renin content; PRC, rats sacrificed 6 hours after injection of mercuric chloride for determination of plasma renin; $HgCl_2$ and UN, rats injected with mercuric chloride and uranyl nitrate, and followed for up to 7 days to determine the course of acute renal failure.

Table 2. Baseline BUN and 24-hour excretion data, groups D to F[a]

	BUN *mg/100 ml*	V *ml/24 hr/100 g body wt*	$U_{Na}V$	$U_{Cl}V$	U_KV
			μEq/24 hr/100 g body wt		
Group D					
RCRC (10)	14 ± 1	6 ± 1	29 ± 10	269 ± 74	511 ± 106
PRC (6)	—	4 ± 1	34 ± 7	376 ± 133	445 ± 122
$HgCl_2$ (10)	15 ± 1	4.0 ± 0.5	39 ± 11	354 ± 56	536 ± 76
UN (11)	15 ± 1	4 ± 1	31 ± 14	443 ± 75	648 ± 102
Group E					
RCRC (8)	14 ± 1	6.0 ± 0.4	404 ± 55	447 ± 72	672 ± 119
PRC (9)	—	6 ± 1	332 ± 54	351 ± 63	592 ± 91
$HgCl_2$ (12)	15 ± 1	6 ± 1	375 ± 53	461 ± 61	707 ± 94
U.N. (10)	15 ± 1	5 ± 1	356 ± 41	451 ± 56	757 ± 83
Group F					
RCRC (9)	14 ± 1	10 ± 1	2501 ± 278	2714 ± 335	906 ± 160
PRC (7)	—	11 ± 1	2382 ± 215	2601 ± 289	783 ± 128
$HgCl_2$ (10)	15 ± 1	9 ± 1	1748 ± 105	1813 ± 144	573 ± 114
UN (15)	16 ± 1	15 ± 1	3110 ± 295	3203 ± 312	973 ± 92

[a] Values are means ± SEM. Number of animals per subgroup is in parentheses. Refer to text or to Fig. 1 for treatment of groups D, E, and F. Subgroups are: RCRC, rats sacrificed for determination of renal cortical renin content; PRC, rats sacrificed 6 hours after injection of mercuric chloride for determination of plasma renin; $HgCl_2$ and UN, injected with mercuric chloride and uranyl nitrate and followed for up to 7 days to determine the course of acute renal failure.

animals, no blood was drawn for baseline BUN determinations.

Sodium and potassium concentrations were measured by flame photometry, with internal lithium standardization. Urinary chloride was measured by titration, with a direct-reading Corning chloride meter. BUN was measured with a General Diagnostic BUN-Strate Kit, based on a method described by Weatherburn [11]. Serum creatinine concentration was measured with a colorimetric method [12]. Methods for RCRC and PRC were described in recent publications of this laboratory [13, 14]. Briefly, renin-containing samples (homogenized renal cortex, plasma) were added to rat renin substrate [15] and incubated at 37° C in the presence of inhibitors of converting enzyme and angiotensinases [16]. In-

cubations were stopped by cooling to 4° C, and the generated angiotensin I was measured by radioimmunoassay. RCRC was expressed in nanograms of angiotensin I per hour of incubation per milligram wet wt of renal cortex (ng AI/hr/mg), and PRC in nanograms of angiotensin I per hour of incubation per milliliter of plasma (ng AI/hr/ml).

Student's t test was used to assess the statistical significance of observed differences. Because of multiple groups and subgroups of different numbers, only P values less than 0.01 were considered significant in some comparisons [17].

Results

Baseline BUN concentration, urine flow, and electrolyte excretion of all rats for the 24 hours preceding the experiments are summarized in Tables 1 and 2. Within all groups, the subgroups (for example, rats sacrificed for baseline RCRC determination) were comparable. Animals drinking tap water and eating the normal chow at the time of the experiments (groups A, C, and E) were excreting similar amounts of water and electrolytes despite the previous dietary manipulations of groups C and E. Sodium excretion of rats on the low-sodium diet (group D) was an order of magnitude less, and that of rats on the high-sodium diet (group B) an order of magnitude greater than control. Short-term sodium loading in previously sodium-deprived rats (group F) resulted in an intermediate enhancement of $U_{Na}V$, from four to six times the control value.

In 31 rats injected with mercuric chloride, BUN and serum creatinine concentrations were simultaneously determined at the time of sacrifice, 5 to 7 days later. BUN concentration ranged from 15 to 400 mg/100 ml; and serum creatinine, from < 1.0 to 18 mg/100 ml. Rats from all three dietary regimens were included: group D, $N = 7$ (low sodium); groups A, C, and E, $N = 14$ (normal sodium); groups B and F, $N = 10$ (high sodium). The correlation coefficient between BUN and serum creatinine concentrations was 0.97, $P < 0.001$. Similarly, the correlation coefficient between BUN and serum creatinine concentrations was 0.93, $P < 0.001$, in 35 rats (all three dietary groups) sacrificed 5 to 7 days after uranyl nitrate injection. Thus, changes in either serum creatinine or in BUN are equally good reflections of changes in GFR.

Means of baseline RCRC and PRC, of PRC 6 hours after injection, and of peak BUN of the various groups injected with mercuric chloride are shown in Fig. 2. As assessed by peak BUN concentration, severity of ARF was identical in groups C and E, neither of which was significantly different from group A. In comparison, severity of ARF was markedly reduced in groups B and F, and mean peak BUN concentrations of these two groups were not significantly different from each other. These

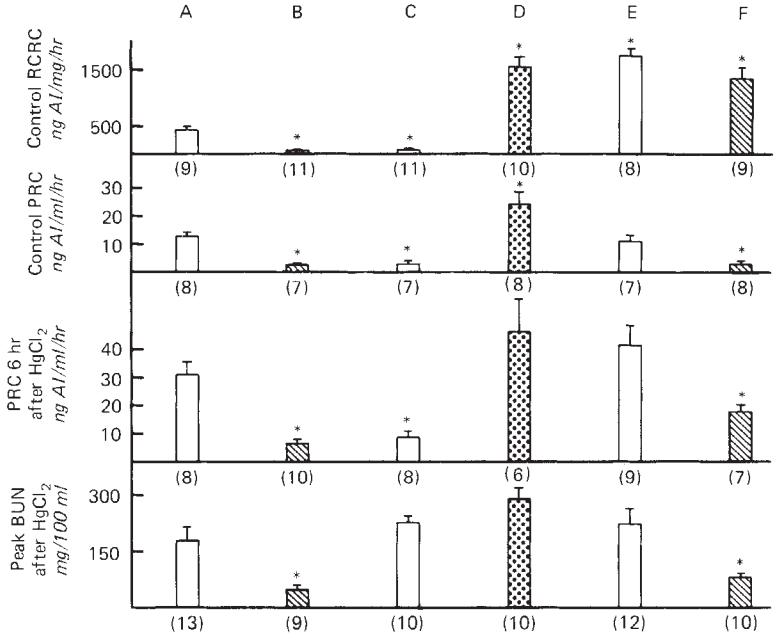

Fig. 2. *Effects of short-term and long-term changes in sodium intake on renal cortical renin content (RCRC), on plasma renin concentration (PRC) before and 6 hours after induction of ARF with mercuric chloride, and on severity of resulting ARF (as assessed by mean peak BUN during the follow-up period).* Number of rats is given in parentheses. *Asterisks* denote values significantly different from control group A ($P < 0.01$).

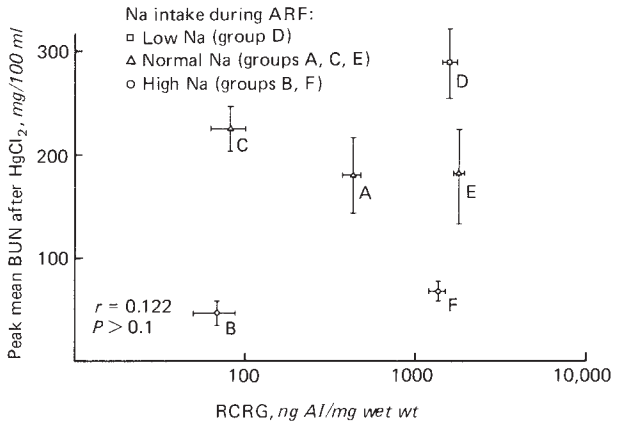

Fig. 3. *Dissociation of renal cortical renin content (RCRC) from the severity of ARF induced by mercuric chloride, as assessed by mean peak BUN following induction.*

observations suggest that the severity of ARF is related to sodium intake at the time of induction of ARF, as groups A, C, and E had normal rat chow and tap water whereas groups B and F had the same rat chow supplemented with sodium chloride to drink.

No pattern was seen between severity of ARF and either baseline PRC or the PRC response to mercuric chloride injection (Fig. 2). For example, both baseline PRC and PRC response were suppressed comparably below normal (group A) in groups B and C, but peak BUN concentration was suppressed only in group B. Thus, neither PRC before the nephrotoxic insult nor the change in PRC during the first few hours after the insult appear to play a role in the severity of ARF that develops.

As can also be seen in Fig. 2, means of RCRC in groups B and C were not significantly different from each other, but both were lower than mean RCRC of group A. Groups D, E, and F were not significantly different from each other, but all had significantly higher RCRC than did Group A. Thus, short-term changes in dietary sodium (for example, 1% sodium chloride rather than tap water for 48 hours, group F; tap water rather than 1% sodium chloride for 4 to 5 days, group C) superimposed on the dietary manipulations of 5 to 6 weeks' duration were not sufficient to modify RCRC, but were sufficient to markedly alter the severity of ARF. This can be seen best in Fig. 3, in which mean peak BUN concentrations are plotted against mean RCRC's of the various groups. If only groups A, B, and D, are considered, the observations confirm those of other investigators [4, 6, 18], that severity of ARF is directly related, in a nearly proportional manner, to RCRC. Exactly the opposite conclusion could be reached, however, if only groups A, C, and F are considered; namely, high RCRC is protective against, whereas low RCRC enhances the severity of, ARF induced by mercuric chloride.

In Fig. 4, mean peak BUN concentrations of the six groups of uranyl nitrate-injected rats are plotted against corresponding mean RCRC's. Comparison of mean BUN concentrations of groups A, B, and F suggests that both short-term and long-term sodium chloride loading were protective against ARF in this nephrotoxic model, but long-term loading was more protective, as mean BUN of group B was significantly less than that of group F. Mean peak BUN concentrations of groups A and D were not significantly different from each other, suggesting that long-term dietary sodium restriction does not enhance the severity of ARF induced by uranyl nitrate. This differed from the result obtained in mercuric-chloride-injected animals (Figs. 2 and 3). Because mean peak BUN of group-A uranyl-nitrate-injected rats was considerably higher, however, than group-A mercuric-chloride-injected rats (282 $\pm$ 28 vs. 180 $\pm$ 36 mg/100 ml, $P < 0.05$), this may mean only that uranyl nitrate at the dose we used causes maximal impairment of renal function even in rats on a normal sodium diet. In any case, RCRC was clearly dissociated from the severity of ARF induced by uranyl nitrate injection.

As suggested above, severity of ARF in the mercuric-chloride-injected rats appeared to be inversely related to dietary sodium at the time of injection. This suggestion is strengthened by data presented in Fig. 5. Mean peak BUN concentrations of groups A to F rats are plotted against corresponding mean 24-hour urinary sodium excretions prior to mercuric chloride injection (sodium intake itself was not monitored). A highly significant inverse correlation was observed ($r = 0.94$, $P < 0.01$). A somewhat lower inverse correlation ($r = 0.91$, $P < 0.05$) was found in the uranyl nitrate model of ARF (Fig. 6).

To study the course of ARF and how it was affected by dietary sodium, we combined for simplification groups A, C, and E (normal intake at the time of injection) and we combined groups B and F (1% sodium chloride rather than tap water at the time of injection). The course of ARF for the three dietary groups injected with mercuric chloride is shown in Fig. 7. In general, mean BUN concentrations at all times measured were inversely related to dietary sodium. Furthermore, the time at which BUN began to decline was inversely related to diet-

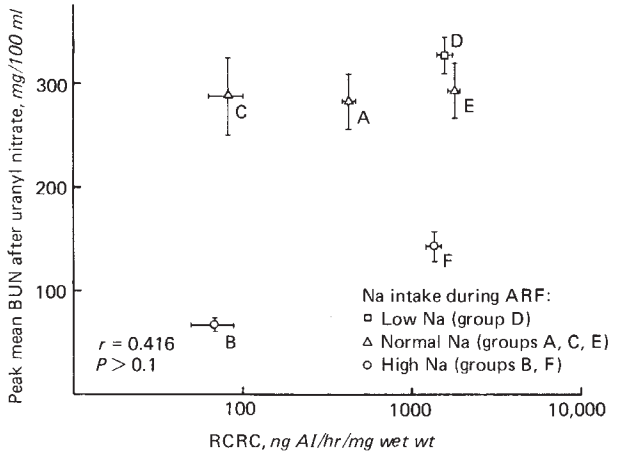

Fig. 4. *Dissociation of renal cortical renin content (RCRC) from the severity of ARF induced by uranyl nitrate injection, as assessed by mean peak BUN following induction.*

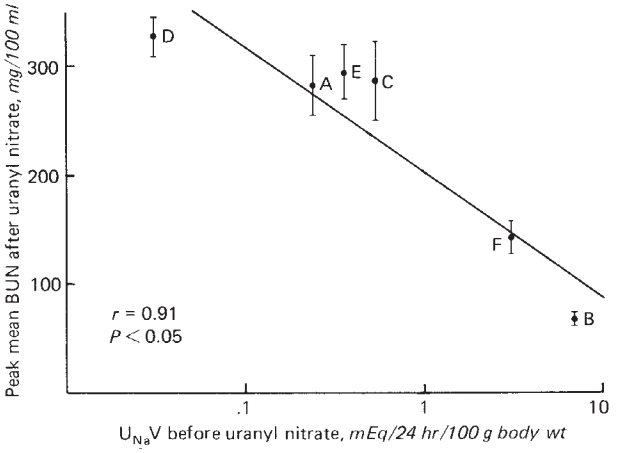

Fig. 6. *Inverse correlation between severity of ARF (mean peak BUN) and mean $U_{Na}V$ during the 24 hours prior to uranyl nitrate injection.* Groups A to F are defined in Fig. 5.

ary sodium. Thus, high sodium both reduces severity and hastens recovery, whereas low sodium enhances severity and delays recovery, in the mercuric chloride model of ARF. The patterns for the same three dietary regimens in the uranyl nitrate model are shown in Fig. 8. Comparison of Figs. 7 and 8 reveals some interesting differences in these two nephrotoxic models. BUN concentration increased more slowly, but to much higher final values, in the uranyl nitrate model. Moreover, in this model, no definite recovery was noticed by day 7. Although mean BUN concentrations of both normal and low sodium groups declined between days 5 and 7, this could be attributed to very high mortality rates, the values at 7 days representing the BUN concentrations of the less severely affected animals. Ex-

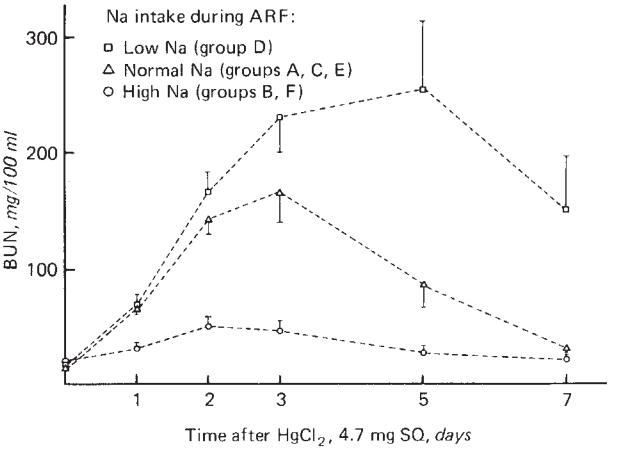

Fig. 7. *Pattern of ARF, as assessed by BUN following injection with mercuric chloride.* Low, normal, and high sodium refer to dietary sodium before and after injection.

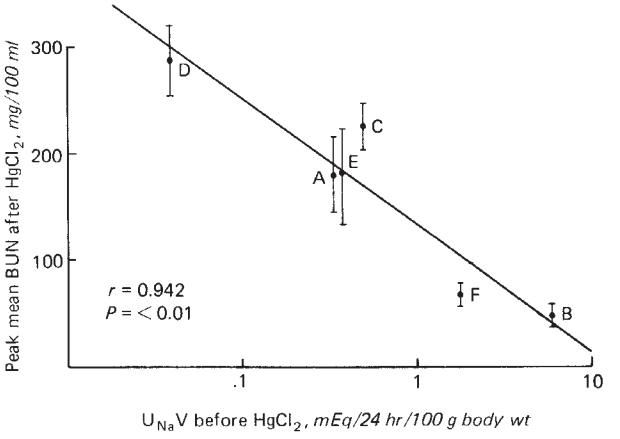

Fig. 5. *Inverse correlation between severity of ARF (mean peak BUN) and mean $U_{Na}V$ during the 24 hours prior to mercuric chloride injection.* Group D was on a low-, groups A, C, and E on a normal-, and groups B and F on a high-sodium diet at the time of injection and thereafter. Correlation coefficient and *P* value were from linear least-squares regression analysis.

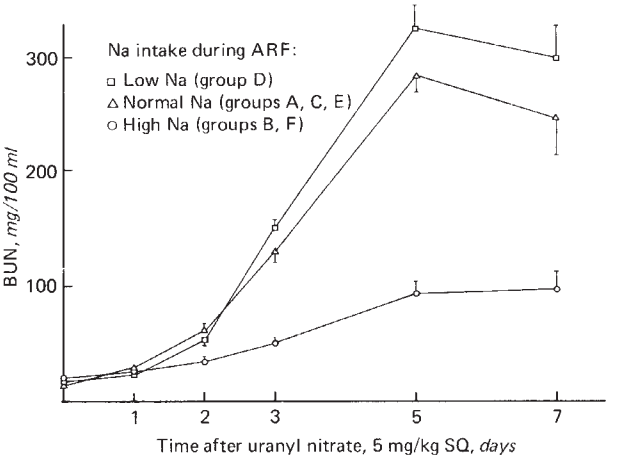

Fig. 8. *Pattern of development of ARF, as assessed by BUN, following injection of uranyl nitrate.* Low, normal, and high sodium refer to dietary sodium before and after induction of ARF.

cluding the sacrificed rats, mortality rates for animals on low-, normal-, and high-sodium intakes were 7/10, 11/35, and 2/19 for mercuric chloride-injected rats, and 8/11, 15/31, and 2/27 for the uranyl-nitrate-injected rats. Finally, in contrast to the mercuric chloride model, differences between the three dietary groups were minimal for the first 2 days.

Discussion

The renin-angiotensin system (RAS) has been postulated to play a major pathogenic role in both the initiation and the maintenance phases of experimentally induced ARF [6, 18–25]. Indeed, factors known to enhance the activity of the RAS, such as fluid deprivation and/or low sodium intake, enhance the severity of myoglobinuric ARF [8, 26], and suppression of the RAS achieved by long-term sodium chloride or potassium chloride loading, protects against ARF induced by the administration of glycerol, dichromate, mercuric chloride, or uranyl nitrate [1–6]. Suppression of plasma renin, however, does not attenuate the severity of experimentally induced ARF in rats, as shown in this study (groups A vs. C) and in others [27]. Neither does any one of a variety of manipulations, all of which block the effects of circulating renin: renin immunization [18]; active and passive angiotensin II immunization [28]; administration of SQ 20,881, an inhibitor of conversion of angiotensin I to angiotensin II [29, 30]; administration of P113, a competitive inhibitor of angiotensin II [29, 30]. Taken together, these observations exclude PRC itself as a pathogenic factor, but they do not exclude intrarenal renin, via local production and action of some species of angiotensin (I, II, or III [31]) at sites inaccessible to antibodies and pharmacologic inhibitors. Because this site might be intracellular [31, 32], and there are no techniques available for directly measuring activity of the RAS at this site, RCRC is currently used as an index. Despite the lack of a proven correlation between RCRC and activity of the RAS at the critical site, only RCRC determinations are available to support the hypothesis that intrarenal renin activity is directly related to the severity of experimentally induced ARF [4, 6, 18].

This study clearly demonstrates that up to 20-fold changes in RCRC have little effect in influencing the severity of ARF, as judged by peak BUN values, in two nephrotoxic models. In fact, this study shows for the first time that rats with high RCRC (four times control value) can be protected against ARF induced with mercuric chloride or uranyl nitrate, by short-term increases in sodium intake and/or excretion. Moreover, RCRC depletion itself failed to protect against ARF, in the absence of increased sodium intake and/or excretion (groups A vs. C). This observation is strengthened by our previous reports that the two kidneys of Goldblatt rats, despite large differences in RCRC, exhibit equal degrees of ARF in response to injections of glycerol [13] or mercuric chloride [33]. The fallacy of assuming a casual relationship between changes in RCRC and severity of ARF is readily apparent in Fig. 3, enabling one to argue either that increases in RCRC attenuate or, conversely, that increases in RCRC enhance the severity of ARF induced by mercuric chloride, depending upon the groups considered.

When the basal PRC, the PRC response to mercuric chloride, and the RCRC are considered together, some interesting features are noted. Though good correlations have been observed between renin release and RCRC [34–36], Fray [37] has suggested that renin release is a function of both RCRC and the sodium intake at the time of stimulated release. If it can be assumed that PRC reflects renin release, the fact that both PRC and the PRC response to mercuric chloride were suppressed in groups B and C is not surprising in view of their markedly suppressed RCRC. On the other hand, the sodium-intake factor might be the explanation for differences in PRC and PRC response to mercuric chloride in groups D to F, despite very similar RCRC. With respect to ARF, though the severity does seem to parallel the PRC response to mercuric chloride in groups D to F (Fig. 2), it is clear from examining groups B and C that severity is completely independent of either PRC or the PRC response to mercuric chloride.

On the other hand, sodium chloride intake and/or excretion is a good predictor of the severity of ARF in both these nephrotoxic models, regardless of the status of the RAS. Thus, a clear separation of the independent effects of sodium chloride on RCRC and on the severity of ARF has been demonstrated in two nephrotoxic models.

The precise reasons for the differences between the two nephrotoxic models, revealed by comparing Figs. 7 and 8, are not clear. In the uranyl nitrate model, BUN increased more slowly, but eventually to much higher levels, and there was no clear-cut recovery during the observation period. Previous work has shown that increased permeability of the tubular basement membrane, with associated backleak of glomerular filtrate, is a major component of the uranyl nitrate model, at least during the maintenance phase [38–40]. In contrast, an element of tubular obstruction is present in the low-dose mercuric chloride model [41]. These differences may be responsible for the different patterns of development of ARF in the two models. There were also

differences between the two models of ARF with respect to the influence of sodium diet. In Fig. 7, the mercuric chloride model, there is a clear separation of groups on low-, normal-, and high-sodium diets. Not only was the severity of ARF ameliorated in the high-sodium group, there was a striking inverse relationship between sodium diet and the time at which recovery began. This suggests that one of the important consequences of increased sodium intake is to hasten recovery and shorten the maintenance phase in the mercuric chloride model. The same may be true in the glycerol model, as Thiel et al [42] found no difference between control rats and rats drinking 1% sodium chloride during the first 4 hours following injection. The latter rats were characterized, however, by a quick recovery in that there were marked differences between them and the controls 24 hours after injection. In contrast to both the mercuric chloride and glycerol models, in the uranyl nitrate model, no significant differences in BUN values were observed between the low-, normal-, and high-sodium-diet groups at the end of 24 hours, and a clear separation between the three groups was achieved only by the second or third day. This suggests that sodium intake exerts its effects during the maintenance phase of ARF in the uranyl nitrate model.

Continued high-sodium diet after the induction of ARF, as in groups B and F of this study, might be necessary to confer protection. This may account for the failure of Flamenbaum et al [27] to observe protection after 30 hours of 1% sodium chloride in the myoglobinuric model of ARF, as the rats in this study were given water after the glycerol injection. This factor of sodium intake following induction of ARF might also account, in part, for the somewhat less complete protection seen in rats drinking sodium chloride short-term (group F, Fig. 6), as these rats were less likely to have achieved a steady-state with respect to sodium balance.

The mechanism by which the level of sodium intake modifies the maintenance phases of these two nephrotoxic models of ARF, independently of the activity of the RAS, remains to be clarified. It is not unreasonable to assume that a state of persistent diuresis and natriuresis would be likely to minimize tubular obstruction and increase nephrotoxin clearance, thus decreasing renal accumulation of nephrotoxin and attendant necrosis [43] and back-leak of tubular fluid. Some suggestive evidence for this postulate has been adduced recently [44], with respect to the mercuric chloride model. Other workers have advanced a similar explanation for the protective effects of sodium chloride, mannitol, or prostaglandin administration in both norepinephrine and methemoglobin-ferricyanide models [45–48]. It is possible, however, that there are other, not yet clarified, mechanisms by which increased sodium chloride intake confers protection [49–51]. Whatever the mechanism(s), we wish to emphasize that in contrast to previous reports in the literature [1–6, 27], our results demonstrate that long-term sodium chloride loading is not necessary for protection, and that substantial protection can be achieved in both models of ARF by short-term (48 hours) increases in sodium chloride intake. This observation, taken with the findings of others [20, 45, 47, 48, 52], may have important clinical implications.

In conclusion, our experiments demonstrate that the effects of sodium chloride intake on activity of the RAS are completely independent of its effects on severity or course of ARF in two nephrotoxic models (mercuric chloride, uranyl nitrate). Although our results cannot exclude an effect during the early initiation phase, the effects of sodium chloride in both models seem to be predominantly expressed during the maintenance phase, and seem to consist of attenuating the severity of (in both models), and hastening the recovery from (in the mercuric chloride model), acute renal failure. Finally, the protective effects can be achieved with increases in sodium chloride intake of 48 hours' duration, regardless of increased activity of the RAS.

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